

**Research Article**

## **PHYTOCHEMICAL SCREENING OF LEAVES EXTRACT OF *MORINGA OLEIFERA* GROWN AT UNIVERSITY FARM GAYA LOCAL GOVERNMENT AREA**

**\*Yahaya S. M., Fagwalawa L. D. and Nabila H. M.**

*Department of Biology, Kano University of Science and Technology, Wudil P. M. B, 3244*

*\*Author for Correspondence*

### **ABSTRACT**

Analysis of leave extract of *Moringa oleifera* plant grown at University farm Gaya was carried out to determine its nutritional values and presence of phytochemicals of medicinal values. The analysis spans a period of Six month between September, 2015 to February, 2016. The leaves extract from *Moringa oleifera* were screened qualitatively and quantitatively to determine the moisture, ash, fat, carbohydrate and some important medicinal phytochemicals. The screening was achieved by serial extractions, using ether, ethanol and water as solvents, the phytochemicals were qualitatively identified in standard form. Moisture and ash content were determined as  $95.510 \pm 0.212\%$  and  $1.075 \pm 0.035\%$  respectively. While fat and carbohydrate content were found to be  $0.770 \pm 0.099\%$  and  $2.245 \pm 0.130\%$  respectively. The phytochemicals of medicinal values identified, included tannins, cardiac glycoside, flavonoid, chlorogenic acid, anthraquinone, saponnin steroids triter perinoids and alkaloids. The presence of these phytochemicals in the extract indicated possible preservative and curative property in the *Moringa oleifera* leaves. This finding could go a long way in standardizing *Moringa oleifera* use for herbal medicine. The finding will also provide baseline information for further research.

**Keyword:** *Ash Content, Moisture Content, Moringa oleifera, Phytochemicals, Screening*

### **INTRODUCTION**

*Moringa oleifera* a member of the family Moringaceae is the most widely cultivated species of monogenetic family (Fahey *et al.*, 2005). It grows well in a hot semiarid and humid region and in a well-drained sandy and loamy soil (Fahey *et al.*, 2005). It is a fast growing evergreen deciduous tree that usually grows as high a 9cm with a soft, white wood and with a corky bark. It consists of different parts that are the leaf, flower, pod, seed, stem and root. It can survive in harsh climatic condition including destitute soil without being much effected by draught (Morton, 1991). It can tolerate wide range of rainfall requirements estimated at 250mm and maximum at over 3000m and a PH of 5.0-9.0 (Morton1991). Its trunk is soft, white corky and branches being a gummy back with innately compound leaves bearing several small leaf lets. The leaf has a long main axis (30-75cm) and jointed branch. The leaflets are glabrous and entire the leaf are finally evergreen (Nadakarni, 1976).

*Moringa* is now been recognized as one of the most nutritional crops grown. The leaves contain higher amount of carotene than carrots, and higher in protein content than peas, more iron than spinach. The *Moringa* plant is now regarded as vital sources of nutrition in the world (Palade and Chang, 2003), as almost every part of the tree can be used for food or has some beneficial properties (Abdul 2007; Chuang *et al.*, 2007). The leaves especially young shoots can be eaten as greens, in salads, vegetable curries, and as pickles. The leaves can be eaten fresh, cooked or stored as dried powder for few months without loss of nutritional value. The leaves are considered to offer great potential to the nutritionally risk individuals as it serves as supplement of protein and calcium its leaves have the calcium equivalent of four times that of milk, the vitamin C content is seven times that of orange while its potassium is three times that of banana, iron is three times of spinach, four times of vitamin A in carrot and two times of protein in milk. Indian has been using it as a regular component of conventional tables for nearly 5000 years (Anwar *et al.*, 2007). Studies by Rajangam (2001), Fahey (2005) and Ogbunugafor *et al.*, (2011) showed that *Moringa* leaves contain low calories and negligible quantities of utilizable energy hence they are ideal for obese people who can satisfy their appetite without consuming much carbohydrate.

## Research Article

Abdul (2007), *Moringa oleifera* leaves are known as excellent sources of nutrition and natural energy booster. It lowers blood pressure and it's a sleep aid (Fugile, 2001). For pregnant and breast feeding woman, moringa leaves can do much to preserve the mother's health and pass on strength to the foetus (Price, 1995). Moringa tree products such as natural antibiotics aid in child birth in treating liver disorder and many others uses. With the abundance uses of *Moringa* there is a great need to know the phytochemical composition of Moringa thereby making retrace for further uses. Ogbunugafor *et al.*, (2011) reported that *Moringa oleifera* is becoming more acceptable in Nigeria because of its nutritional and medicinal values. Therefore, this research was aim at finding the phytochemical composition of the Moringa with the objectives of comparing them, knowing them individually, and relating the presence of the phytochemicals to the medicinal value of the leaf.

## MATERIALS AND METHODS

The leaves of *Moringa oleifera* were collected from farmers around Gaya Local Government Area. The plant was identified by extension worker at Kano Agricultural and Rural, Development Agency (KNARDA) Gaya Zone 3.

### Determination of Moisture Content in *Moringa oleifera* Leaves

A clean dry petridish was weighed as (W1) and 5g of the sample was placed on the petridish and weighed as (W2). The petridish containing the sample was then placed on an oven at 120°C. After 1 hour, the petridish was removed and cooled in a desiccator for 30 minutes and weighed.

### Sample Handling

The study plant was dried at shady temperature of 28°C and made into powder, by mortar and pestle. The powdered samples were then stored in air tight containers and properly labeled, and were used to make the tests for alkaloid, saponin, and tannin, while the phytochemical analysis were carried out using standard procedures.

### Determination of Ash Content in *Moringa oleifera* Leaves

A silica dish was cleaned, dried and cooled (in a desiccator) and weighed as (W1). About 2g of the test substance was weighed accurately into the dish and weighed as (W2).

The silica dish was then placed on an oven for 30 minutes. It was then transferred to an electric burner for 2 hours until fully ash. Nitrate grey colour of ash. The dish with ash was cooled in desiccators and weighed as (W3).

$$\text{Calculation: } \% \text{ ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100\%$$

### Determination of Fat Content in *Moringa oleifera* Leaves

Ten (10) g of the sample was weighed accurately. The sample was poured into a tap running separating funnel and 25ml of petroleum ether was added to the separator and it was shaken vigorously and allows setting. A cleaned, dried conical flask was weighed as (W1). It was kept below the ether layer. The clean extracts were runoff into the conical flask. The extraction was repeated twice with 25ml of diethyl ether. Extract was dried until the solid particles dissolved and the mixture becomes brown or violet in colour. The tube was taken off and cooled rapidly and weighed.

$$\text{Calculation: } \% \text{ Fat} = \frac{W_2 - W_1}{W} \times 100\%$$

### Determination of Carbohydrate Content

Carbohydrate content of the *Moringa oleifera* was determined using the standard method described by (AOAC) 1984.

Percentage (%) available carbohydrate = 100% – (% moisture + % ash + % fat + % protein)

### Determination of Tannin

Three grams of the powdered sample was boiled in 50mls of distilled water for 3 minutes on the heater mantle. The mixture was filtered and used to carry out the following tests for tannins

## **Research Article**

### **Ferric Chloride Test**

A portion of the water extract was diluted with distilled water in a ratio of 1:4 and a few drops of 10% ferric chloride were added. A blue color was observed which indicated the presence of tannins (Fugile, 2001).

### **Test for Glycosides**

#### *General Test for Glycosides*

In this test 2.5mls of dilute H<sub>2</sub>SO<sub>4</sub> was added to 5mls of extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH. To this 5mls of Fehling's solution A was added a red precipitate or (reducing sugar) was observed and indicated the presence of glycosides.

### **Test for Anthraquinone**

In this 0.5g of powdered sample was boiled with 10mls of 10% hydrochloric acid for 2 minutes. The extract was filtered. To the filtrate an equal volume of chloroform was added. The test tube was inverted for a couple of times avoiding vigorous shaking. The solution was transferred into a separating funnel and the two layers were allowed to separate. The lower chloroform layer was poured into a clean test tube and 10% ammonia solution was added and shaken. The two layers were again allowed to separate. A bright pink color was observed in the upper layer, aqueous layer this indicates the presence of free combined Anthraquinones (Fugile, 2001). To one mg of the extract, 2ml of 25% ammonia solution was added and shaken. (A cherish red solution was observed and this indicated the presence of a compound (Anthraquinones).

### **Test of Saponins**

Ethanol (95%) ethanol was added to a small quantity of the powdered sample, and boiled. The mixture was filtered and 2.mls of the filtrate was added to 10mls of distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 seconds; it was allowed to stand for over 30 minutes. Honey – comb froth was observed, this indicated the presence of saponin.

### **Test for Flavonoids**

#### *Extraction*

Five grams of the powdered sample was completely detanned (Ogbunugafor *et al.*, 2011) with acetone on a water bath. The mixture was filtered and the filtered used for the following tests.

#### *Lead Acetate test*

Ten percent lead acetate was added to 5mls of the detanned water extract, a coloured precipitate was formed which indicated the presence of flavonoid.

#### *Sodium hydroxide*

Five mls of 10% sodium hydroxide was added to an equal volume of the detanned water extract. This resulted into the formation of a solution, this indicated the presence of flavonoids.

#### *Ferric Chloride Test*

Two mls of the detanned water was diluted with distilled water in a ratio of 1:4 and a few drops of the 10% phenolic solution added. The mixture showed a green solution which indicated the presence of ferric chloride.

### **Test for Alkaloids**

Ten grams of the powdered sample was taken in a small beaker and a solution of ammonia solution added in a quantity sufficient to just moisten it, it was allowed to stand for 10 minutes after through mixing of the contents. Sufficient quantity of the chloroform and ethanol (1:1) was added just to soak and suspend the powder. The mixture was allowed to stand for 20 minutes with occasional stirring using a glass. The mixture was filtered through a plug of cotton wool the mare was washed twice with 2mls of chloroform and the washing was combined with the filtrate. The bulked filtrate was concentrated to dryness without overheating.

The residue was cooled and dissolved in 5mls of chloroform. The chloroform solution was transferred to a separating funnel and shaken with dilute sulphuric acid. The two layers were allowed to separate; the chloroform lower layer was drained off and discarded until the upper acid layer was colorless. The acid layer was made completely alkaline by adding strong ammonium solution (this tested by adding indicator

### Research Article

paper). The extraction with the chloroform extracts was retained and evaporated to dryness (Ogbunugafor et al., 2011).

The residue was dissolved in 3mls of ethanol and following tests were carried out, it was neutralized with dilute sulphuric acid.

#### Test for Coumarins

One milligram of the extract was dissolved in 2mls of water the solution was divided into proportions to the first portions .5ml 10% of ammonia solution was then added. The second acted as reference. The first solution was subjected to an ultra violet light; an intense florescence was formed. This showed the presence of coumarins and its derivatives.

#### Steroids Triter Penriods Procedure Test

One milligram of dried extracts was dissolved in 0.5mls of acetic anhydride, to which 0.5mls of chloroform was added. The solution was pipetted into a dry test tube and then 1ml of H<sub>2</sub>SO<sub>4</sub> was added at the bottom of the test tube.

This resulted in the formation of a brown ring between the compounds, indicating the presence of steroids triter penoids.

## RESULT AND DISCUSSION

### Moisture Content of Moringa Leaves Extract

Moisture content ranges from (95.510 ± 0.212%). Sengupta (1980) reported that moisture was upto 87%, among other leaves, such as *Talinum triangulare* had 91.6% which may be due to large number of cell sap they possess.

The range of moisture content obtained in the present study agree with the result of Tindall (1988) who reported the moisture content of moringa leaves as 90 – 93%. While for *Vernonia amyglalina* it was 87.7% as reported by Sengupta (1980).

The minor difference recorded may be attributed to the differences in the period of the experimental work (Table 1).

### Ash Content of Moringa Leaves Extract

Ash content of (1.075 ± 0.035%) was recorded (Table 1). This was comparatively low when compared to values obtained in previous research with ash content value of 7.64%, 7.93%, 3.8% and 10.6% respectively (Busani et al., 2001).

Other leaves like *Vernonia amyglalina* was observed to be (2.5%) which may be due to higher content of minerals. The values obtained fall due to the addition of water in the process. Ash content of moringa leaves was lower than that of some leafy vegetables commonly consumed in Nigeria. This include *Talinum triangulare* (20.05%), *Acalypha marginata* (15.68) but they compare favourably with some other vegetables such as *Occimum gratiimbium* (8.00%) and *Hibiscus esculent* (8.00%) (Akindahunsi et al., 2005).

### Fat Content of Moringa Leaves Extract

Fat content from the result (Table 1) was (0.770 ± 0.099%). When compared with other values it is lower. Moderate amount of fat indicates that the vegetable is not a source of lipid accumulation which can cause arteriosderosis, aging (Antia et al., 2006).

The fat content obtained from Moringa leaves by Ifon et al., (1979) was lower than that reported in *Talinum triangulare* (5.90%), *Baseila alba* (8.71%), *Amaranthus hybridus* (4.80%), *Acalypha tacemosa* (6.3%).

### Carbohydrate Content of Moringa Leaves Extract

The results obtained from the proximate analysis of the *Moringa oleifera* leaves establishes that they can be ranked as carbohydrate leaves due to their relatively high carbohydrate content. The result showed that carbohydrates have the high percentage in Moringa leaves (2.245 ± 0.130%) this was similar to the result obtained by Oke (1986).

The result however, was lower than that of Sengupta (1980) who reported (4.5%) carbohydrate content from leave extract of Moringa leaves (Table 1).

**Research Article**

**Table 1: Proximate Composition of *Moringa oleifera* Leaves Extract with the % Composition of Moisture, Ash, Protein, Carbohydrate and Total Solid**

S/N	Moisture AVG SD	Ash AVG SD	Fat AVG SD	Protein AVG SD	% Total Average Solid AVG SD	% Carbohydrate AVG SD
1A	94.780 ± 0.622	0.925 ± 0.025	0.490 ± 0.014	1.563 ± 0.0442	5.330 ± 0.77	2.243 ± 0.130
2B	94.310 ± 1.344	1.025 ± 0.177	0.470 ± 0.014	1.250 ± 0.00	5.690 ± 1.344	2.945 ± 1.153
3C	94.820 ± 1.018	1.075 ± 0.035	0.770 ± 0.099	1.875 ± 0.00	5.180 ± 1.018	1.457 ± 0.884
4D	94.310 ± 0.268	1.025 ± 0.035	0.610 ± 0.014	1563 ± 0.442	5.190 ± 0.976	1.993 ± 1.467
5E	95.290 ± 0.382	0.750 ± 0.282	0.760 ± 0.057	1.875 ± 0.884	4.710 ± 0.382	1.325 ± 1.029
6F	95.260 ± 0.368	0.550 ± 0.071	0.680 ± 0.028	2.213 ± 0.477	4.740 ± 0.368	1.298 ± 0.944
7G	94.600 ± 0.396	0.850 ± 0.071	0.710 ± 0.014	1.563 ± 0.442	5.400 ± 0.396	2.268 ± 0.117
8H	95.510 ± 0.212	0.725 ± 0.035	0.640 ± 0.056	2.500 ± 0.00	4.590 ± 0.212	0.725 ± 0.120

**Phytochemical Test of *Moringa Leaves Extract***

The result showed some of the phytochemicals found in the *Moringa oleifera* leaves extract. They are tannins, saponins, glycoside, flavonoids, alkaloids, anthraquinones, coumarins and steroids triter penoid. The Table as well revealed the quantitative estimation of the phytochemicals found in the extract. The estimation showed the presence of more tannin, flavonoids and cardiac glycosides (Table 2).

**Table 2: Phytochemical Present in *Moringa Oleifera* Leaves Extract**

Phytochemical	Result
Tannins	++
Cardiacglycosides	++
Flavonoids	++
Chlorogenic acid	+
Anthraquinone	+
Saponins	+
Coumarins	-
Steroids Triter Penoid	+
Alkaloids	+

Key: + = Means Present; ++ = More concentration; - = Absent

The result of the present study confirmed that the leaves of *Moringa oleifera* used contained tannin, flavonoids, saponin, anthraquinones, steroids, titer penoids, coumarins and glycosides. However, coumarins, was not present in the extract and therefore agreed with the earlier studies which also found that, not all phytochemicals are present in all plant parts and that those present differ according to the type of the solvent used in the extraction (Abdul, 2007). Flavonoid found was shown to occur among the more concentrated phytochemicals. This was told by (Price 1995).

The result of the present study shows that *Moringa oleifera* is an effective antimicrobial plant, results of the previous studies showed that flavonoid are strong antioxidants, they are also found to be an effective

### Research Article

antimicrobial substances in vitro against a wide array of microorganisms by inhibiting the membrane bound enzymes (Cowan, 1999; Broadhurst and Anderson, 2000) also reported that they possess substantial anti carcinogenic and antimutagenic activities due to their oxidant and inflammatory properties this was also confirmed by (Nandukumar *et al.*, 2008). The Flavonoids are also active in reducing high blood pressure this was reported by Abdul (2007) and Dhawan and Jain, (2005). All these put together suggest the rise of *Moringa oleifera* for anti-microbial activity and anti-hypotensive. Tannins are a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution (Scalbert, 1991), causing local tumors in activating and killing micro-organisms (Cowan, 1999). The presence of the tannin, therefore, is an indication of the property of inactivation and killing microorganism in *Moringa oleifera* and a further suggestion of anti-microbial property in the plant.

Anthraquinones are group of naturally occurring phenolic compounds found in *Moringa oleifera* leaves and tend to have laxative effects. Terpenoids and steroids were also found. Their presence is supported by Cowan (1999) who reported their presence and described them as being active against bacteria such as staphylococcus aureus, and this capable of preventing cancer (Scalbert, 1991), having anti-carcinogenic effects by (Yun, 1996). The presence of saponins also suggest that the leaves of *Moringa* can be used in many ailments like anti-inflammatory, anti-apoptosis etc the presence in the leaf extract and the ailments have all been supported by the literature of Rausch *et al.*, (2006).

The photochemical analysis revealed the presence of alkaloids. These are nitrogen naturally occurring compound. They contain antimicrobial properties. This was supported by Fahey (2005), and that of hypertensive thiocarbamate glycosides in *M. oleifera* by Fahey (2005). This also suggests and contributes to the use of the plant in hypertension. On other hand it was also reported to modify tumorigenesis (Ueno *et al.*, 2009), able to inhibit carbohydrate mediated tumor growth (Nangia Makker *et al.*, 2002) and introduce a stress response and apoptosis in human breast cancer cells. The finding Abdul (2007) shows although there is low level of protein in *Moringa* leaves, however, there is increasing awareness of the importance of the vegetable in maintaining health, particularly in areas where animal protein is scarce.

This study has established the fact that *Moringa oleifera* leaves could be used for the treatment of various ailments. The presence of some phytochemicals in *Moringa oleifera* such as tannin, cardio glycoside, flavonoid, chlorogenic acid, anthraquinone, saponins, steroid and triterpenoids which contain an element of compound which serve as antibiotic or antimicrobial or antibacterial activities in the human bodies. According to Fahey (2005) *Moringa oleifera* leaves are the ailments curative, and therefore, people could use it at primary health care level before seeking help at health facilities. Fahey (2005) also revealed that there are 43 uses of *Moringa oleifera* around the world among which are preventive and curative.

Therefore, from the finding of this study, it could be recommended that *Moringa oleifera* leaf can be used for different drugs production and as well as for primary health care practice.

### REFERENCES

- Abdul DAS (2007).** *Economic Importance of Moringa oleifera in Tafa Local Government Area of Niger State.* NDE Project. (Federal College of ForestryMechanization, Kaduna, Nigeria) 34.
- Anwar F, Latif S, Ashraf M and Gilan AH (2007).** *Moringa oleifera: A food plant with multiple medicinal uses.* *Phytotherapy Research* **21** 17-25.
- Broadhurst CL, Polansky MM and Anderson RA (2000).** Insulin-like biological activity of culinary and medicinal plant aqueous extract in vitro, *Journal of Agricultural and Food Chemistry* **48** 894-54.
- Chuang O, Lee C, Chou J, Murugan M, Shief B and Chen H (2007).** Antifungal activity of crude extracts and essential consumed during drought. Study of rural Fulani, Northeastern Nigeria. *International Journal of Food Sciences and Nutrition* **51**(3) 195-208.
- Cowan MM (1999).** Plant products as antimicrobial agents. *Clinical Microbiology Reviews* **12** 582.
- Dhawan V and Jain S (2005).** Garlic supplementation prevents oxidative DNA damage in essential hypertension. *Molecular and Cellular Biochemistry* **275** 85-94.
- Fahey JW (2005).** *Moringa oleifera: a review of the medicinal evidence for its nutritional, therapeutic and prophylactic properties.* *Trees for Life Journal* **1** 5

### Research Article

- Fuglie I (2001).** The miracle tree: *The Multiple Attributes of Moringa*, (Dakar, Senegal: Church World Service) 45-76.
- Morton JE (1991).** The horseradish tree, *Moringa pterygosperma* (Moringaceae) – a boon to arid lands. *Economic Botany* **45**(3) 318-333.
- Nandakumar V, Singh T and Katulyar S (2008).** Multi-targeted prevention and therapy of cancer by proanthocyanidins. *Cancer Letters* **269** 378-387.
- Nangia –Makker P, Hogan V, Honjo Y, Baccarini S, Tait L, Bressaller R and Raz A (2002).** Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin. *Journal of the National Cancer Institute* **94** 1854-1862.
- Ogbunugafor HA, Eneh FU Ozumba AN, Igwo-ezikpe MN, Okpuzor J, Igwilo IO, Adenekan SO and Onyekwellu OA (2011).** Physio-chemical and anti-oxidant properties of *Moringa Oleifera* seed oil. *In Pakistan Journal of Nutrition* **10**(5) 409-414.
- Palada MC and Chang LC (2003).** Suggested cultural practice of Moringa: AVRDC *International Co-Operators Guide*, (Shanhua, Taiwan, Mardi AVRDC Publication) 545.
- Price M (1995).** *Moringa Tree in Natural Nutrition for Tropics 1*, (Sierra Sunrise Books Missouri, USA) 12-15.
- Rajangam J, Azahakia Manavalan RS, Thangaraj T, Vijayakumar A and Muthukrishan N (2001).** Status of production and utilization of Moringa in Southern India. In: Lowell, J.F. (edition), *The Miracle Tree/ The Multiple Attributes of Moringa*. (CTA, USA).
- Rausch W, Liu S, Gille G and Redad K (2006).** Neuroprotective effects of ginsenosides. *Acta Neurobiologiae Experimentalis (Wars)* **66** 297-375.
- Scalbert A (1991).** Antimicrobial properties of tannins. *Phytochemistry* **30** 303875-3883.
- Caceres A, Saravia A, Rizzo S, Zabala L, Leon ED and Nave F (1992).** Pharmacologic properties of *Moringa oleifera*. 2: Screening for antispasmodic, anti-inflammatory and diuretic activity. *Journal of Ethnopharmacology* **36** 233–237.
- Ueno M, Inano H, Onado M, Murase H, Ikota N and Kagiya T Anzai K (2009).** Modification of mortality and tumorigenesis by tocopherolmono-glucoside (TMG) administered after Xirradiation in mice and rats. *Radiant Research*. 172: 519-524.
- Yun K, Lee Y, Kwaon H and Choi K (1996).** Saponin contents and anticarcinogenic effects of ginseng depending on types and ages in mice. *Zhongguo Yao Li Xue Bao* **17** 293-298.